6TH ANNUAL

PACIFIC RESEARCH DAY & STUDENT RESEARCH COMPETITIONS

PROGRAM & ABSTRACTS

TUESDAY JUNE 1 2004

ORTHO RESIDENT PRESENTATIONS

FACULTY & STUDENT TABLE CLINICS

ADA/DENTSPLY & SENIOR RESEARCH COMPETITIONS

PROGRAM

PRESENTATIONS BY ORTHODONTICS RESIDENTS

12 noon – 2:00 pm

Room 304

12:00 Cory Costanzo, Marie Tolarova, Mirek Tolar, Hee Soo Oh, Diana Grochova and Tripti Pawar
THE G ALLELE AT THE 80TH NUCLEOTIDE OF THE RFC1 GENE IS A GENETIC RISK FACTOR FOR NONSYNDROMIC CLEFT LIP AND PALATE

12:20 A. Kouvaris and S. Baumrind
DIFFERENCES IN PROFILE CHANGE DURING TREATMENT IN EXTRACTION AND NON-EXTRACTION PATIENTS

12:40 Tom Ellerhorst and Sean Carlson
QUANTIFYING MAXILLARY 1ST MOLAR DISPLACEMENT ASSOCIATED WITH NON-EXTRACTION, CLASS II CORRECTION IN A GROWING POPULATION

1:00 Shawn Perce and Sean Carlson
ASSOCIATION BETWEEN PREMOLAR EXTRACTIONS AND THE VERTICAL DIMENSION

1:20 Vernon W. Pollock, Sheldon Baumrind, Robert L. Boyd, Sean Carlson
CLINICIANS' ESTIMATES OF INCIDENCE OF TOOTH LOSS RELATED TO APICAL ROOT RESORPTION

1:40 Eric Axelrode*
RELATIONSHIP OF CEPHALOMETRIC VALUES WITS, SNA, SNB AND ANB TO FACIAL ATTRACTIVENESS

* Will not be presenting

FACULTY POSTERS
& TABLE CLINICS

3:00 – 7:30 pm

Café Pacific

David W. Chambers
DO REPEATED CLINICAL COMPETENCY RATINGS STEREOTYPE STUDENTS?

Valentina A. Khorosheva and Joel A. Cohen
ELECTROKINETICS OF STEALTH® LIPOSOMES

Matthew Hashimoto, Christopher Hatae, Geoff & Joan Lin-Cereghino
CHARACTERIZATION OF KANAMYCIN AS A SELECTABLE MARKER FOR MULTICOPY TRANSFORMANTS IN P. PASTORIS

K. Konopka, A. Lee, J. Monzon-Duller, and N. Düzgüneş
CELL-SURFACE HEPARAN SULFATE AND HIV-1 INFECTION OF DIFFERENTIATED THP-1 CELLS

K. Konopka, B. Dorocka-Bobkowska, and N. Düzgüneş
AMBISOME INHIBITS THE ADHERENCE OF CANDIDA ALBICANS TO HELA CELLS

Midori Obara, Terezie Mosby, HeeSoo Oh, Tripti Pawar, Alex Shaw and Marie Tolarová
OROFACIAL CLEFTS: DO MOTHERS OF CHILDREN KNOW WHY IT HAPPENS? A CASE STUDY

Ninad Patil and Timothy J. Smith
AN EVALUATION OF THE USE OF AN ARTIFICIAL NEURAL NETWORK TO PREDICT THE RISK FOR ADVERSE DRUG REACTIONS FROM LOW-DOSE ASPIRIN CHEMOPROPHYLAXIS

Tripti Pawar, Miroslav Tolar and Marie Tolarová
FACTORS AT PLAY IN CLEFTING. AN EPIDEMIOLOGICAL STUDY SEARCHING FOR VARIOUS FACTORS CAUSING CLEFT LIP AND PALATE ANOMALIES IN CEBU CITY, PHILIPPINES

M. Tolarova, T. Mosby and S. Estupinan-Day
UPDATE ON PRIMARY PREVENTION OF CLEFT LIP AND PALATE ANOMALIES

H.S. Oh, T. Mosby, C. Torfs and M. Tolarova
EPIDEMIOLOGY OF OROFACIAL CLEFTS IN TRELEW AND IN CHILLAN

ADA / DENTSPLY
STUDENT RESEARCH COMPETITION *

5:00 – 7:30 pm

Café Pacific

Sam Christensen, Phillip Fletcher, Tripti Pawar, Dianna Grochova and Marie Tolarová
IMPACT OF PATERNAL METHYLENETETRAHYDROFOLATE REDUCTASE GENE (MTHFR) 677CT MUTATION ON FACIAL CLEFTS

Andrea Delurgio, Nate Overlid, Krystyna Konopka and Nejat Düzgüneş
SUICIDE GENE THERAPY FOR ORAL CANCER: APOPTOSIS OR NECROSIS?

Marcus Kai, Galinna Lin and Marie Tolarová
CHILDREN AFFECTED WITH AN OROFACIAL CLEFT IN JIUJIANG, CHINA: AN EPIDEMIOLOGICAL STUDY

Galinna Lin, Marcus Kai and Marie Tolarová
A STUDY OF FAMILIAL AND ENVIRONMENTAL RISK FACTORS FOR CLEFT LIP AND PALATE, IN JIUJIANG, CHINA

Daniel Martin, Dustin Wirig and Marie Tolarova
EPIDEMIOLOGY OF OROFACIAL CLEFTS IN THREE POPULATIONS

Thao Nguyen and Marie Tolarová
ULTRASOUNDS: A LITTLE INFORMATION CAN GO A LONG WAY

Bakhtiar Ardi Pribadi, Angelle Casagrande and Thomas Indresano
MANDIBULAR FRACTURE SURVEY: A 10-YEAR RETROSPECTIVE AND PROSPECTIVE ANALYSIS

Seth Reder and Claire Garcia
THE USE OF THE INTRA ORAL CAMERA WITH PEDIATRIC PATIENTS IN A PEDIATRIC DENTAL CLINIC

Ryan Savage and Miriam Gochin
DEVELOPING AN ASSAY TO DETECT LIGAND BINDING TO THE COILED COIL DOMAIN OF HIV-1 GP41

Caton State, Nate Overlid, Krystyna Konopka and Nejat Düzgüneş
NOVEL TRANSFECTION REAGENTS FOR GENE THERERAPY OF ORAL CANCER

* Sponsored in part by Western Dental Services, Inc.

SENIOR RESEARCH COMPETITION *

5:00 – 7:30 pm

Cafe Pacific

Basma Fallah, JoMarie Monzon-Duller, Krystyna Konopka and Nejat Düzgüneş
SERUM-RESISTANT HSVTK/GANCICLOVIR GENE THERAPY IN ORAL CANCER CELLS

Joseph R. Kolody, David R. McDonough and Nejat Düzgüneş
THE EFFICACY OF ECOTRU AND TRICIDE DISINFECTANTS ON HARD SURFACES OF DENTAL UNITS IN A LARGE DENTAL SCHOOL CLINIC

David Martin, Dallen Phillips and Leigh Anderson
PARASYMPATHETIC VASODILATATION IN RAT SUBMANDIBULAR GLANDS: CONTRIBUTION OF ENDOTHELIUM-DERIVED RELAXING FACTORS

Reza Riahi, JoMarie Monzon-Duller, Krystyna Konopka and Nejat Düzgüneş
PROTAMINE ENHANCES TRANSFERRIN-LIPOPLEX-MEDIATED GENE DELIVERY AND HSV-*tk*/GANCICLOVIR-MEDIATED CYTOTOXICITY IN ORAL CANCER CELLS

* Sponsored in part by Western Dental Services, Inc.

ABSTRACTS

ORTHODONTICS RESIDENTS

THE G ALLELE AT THE 80TH NUCLEOTIDE OF THE RFC1 GENE IS A GENETIC RISK FACTOR FOR NONSYNDROMIC CLEFT LIP AND PALATE

Cory Costanzo[1], Marie Tolarova[2], Mirek Tolar[3], Hee Soo Oh[1], Diana Grochova[2] and Tripti Pawar[3]

[1]Orthodontic Residency Program and [2]Craniofacial Genetics Laboratory, Department of Orthodontics, School of Dentistry, University of the Pacific, 2155 Webster Street, San Francisco, CA 94115; [3]Pediatric Clinical Research Center, PCRC Core Laboratory, Children's Medical Center, UCSF, 505 Parnassus Avenue, San Francisco, CA 94143

INTRODUCTION: Nonsyndromic cleft lip and palate (NCLP) is among the most common congenital anomalies with a prevalence of approximately 1 in 700 live births. The etiology of NCLP consists of both environmental and genetic factors, including folic acid intake and folic acid metabolism. Because of their suspected importance, genes involved in the metabolism of folic acid have been considered as candidate genes for the etiology of NCLP.

OBJECTIVES: Our study focused on a possible role of the reduced folate carrier 1 (RFC1) gene, which is involved in transporting folate across the cell membrane. We studied the polymorphism at nucleotide 80 (A80G).

METHODS: We investigated a sample of individuals affected with NCLP (n=97) and a sample of unaffected individuals (n=76) from the same location. Cases and controls were identified during Rotaplast medical missions at Roosevelt Hospital in Guatemala City, Guatemala. Diagnosis of cleft was determined by physical examination of each individual. DNA was isolated from dry blood spots on filter paper. RFC1 A80G genotypes were established by PCR amplification and single nucleotide conformational polymorphism detection using polyacrylamide gel electrophoresis.

RESULTS: A significant difference (χ^2, p=0.0157) was found in genotype distribution between cases and controls. In cases, 19.6% of individuals had A80/A80 genotype, 34.0% had G80/G80 genotype, and 46.4% were heterozygotes (A80/G80). Proportions of genotypes in controls were 39.5% A80/A80, 25.0 % G80/G80, and 35.5% A80/G80. The A allele frequency was 0.427 for cases and 0.572 for controls, while the G allele frequency was 0.573 for cases and 0.428 for controls (χ^2, p=0.0104, Odds ratio=0.56; 95% Confidence limits: 0.35, 0.88).

CONCLUSION: Results of this study suggest that the G allele in nucleotide 80 of the RFC1 gene contributes to the etiology of NCLP in Guatemala.

The fieldwork for this study was supported by Rotaplast Intl., DNA analysis was supported by The Smile Train/PSEF grant.

DIFFERENCES IN PROFILE CHANGE DURING TREATMENT IN EXTRACTION AND NON-EXTRACTION PATIENTS

A. Kouvaris[1] and S. Baumrind[2]

[1]Orthodontics Residency Program and [2]Department of Orthodontics, School of Dentistry, University of the Pacific, 2155 Webster Street, San Francisco, CA 94115

OBJECTIVE: To investigate differences in the anterior hard and soft tissue changes observed during treatment between class I and class II patients treated with and without four bicuspid extraction.

METHOD: This initial study sought to identify cephalometric variables whose change during treatment differed as a function of four bicuspid extraction. A new random sample was collected retrospectively in the practice of a single experienced orthodontist. The sample included 48 growing subjects equally balanced (i.e., 24/24) for extraction/non-extraction, Class I/Class II and Male/Female. All subjects had been treated using the full bonded Edgewise appliance. Treatment-associated changes in seventeen hard and soft tissue linear and angular measures were computed from triple determined landmark coordinates.

RESULTS: Only seven of the seventeen measures had mean differences in change during treatment in excess of one degree or one millimeter. These were Naso-Labial Angle2 (Δ3.13°), Interincisal Angle (Δ3.04°), Lower 1 to NB Angle (Δ2.36°), Naso-Labial Angle1 (Δ2.00°), Lower 1 to NB Distance (Δ1.42mm), Subnasale to Soft Tissue B point (Δ1.07mm), and Subnasale to Upper Lip Anterior (Δ1.04mm). Only the differences in change for Lower 1 to NB Distance were statistically significant ($t = 2.44$, $p<0.02$). For the other six measures, inter-individual variability was too great to be sure the observed differences in mean were beyond the probability of chance. It was noted that among the extraction cases (n=24), the retraction of the lower incisor was statistically significantly but mildly associated with greater retraction of the lower lip ($r = 0.45$, $p<0.03$). This association was not observed among the non-extraction cases ($r = 0.07$, $p< 0.76$).

CONCLUSION: In general, differences in change during treatment between extraction and non-extraction patients did not appear to be sufficiently large to have substantially different effects on the pretreatment profile.

QUANTIFYING MAXILLARY 1ST MOLAR DISPLACEMENT ASSOCIATED WITH NON-EXTRACTION, CLASS II CORRECTION IN A GROWING POPULATION

Tom Ellerhorst[1] and Sean Carlson[2]

[1]*Orthodontic Residency Program and* [2]Department of Orthodontics, *School of Dentistry, University of the Pacific, 2155 Webster Street, San Francisco, CA 94115*

This retrospective study looked at pre and post-treatment lateral cephalographs to quantify the displacement of maxillary first molars within the maxilla. Displacements of the MB root apex and MB cusp tips, as well a the change in the molar angle were recorded through the use of modified Bjork maxillary anatomic superimpositions and the establishment a coordinate reference plane based on the palatal plane of the pre-treatment headfilm. Three distinct groups of subjects (1 Begg sample and 2 Edgewise samples) treated by three separate clinicians were used to develop a sample totaling 144 subjects, all younger than the age of 18 years upon completion of treatment. A special emphasis was placed on class II non-extraction treatment. The results demonstrated that the molars were displaced in a variety of directions during successful treatment of these class II non-extraction cases. In all cases, extrusion occurred. In the Begg sample, this extrusion was accompanied by minimal mesial molar movement (0.04mm at the apex, 0.33mm at the cusp tip). In one of the Edgewise samples, the extrusion was accompanied by molar distalization (-2.48mm at the apex, -0.69mm at the cusp tip), while the other Edgewise sample demonstrated extrusion and mesialization (0.59mm at the apex, 3.52mm at the cusp tip) of the maxillary first molar. In all cases, the molar angle decreased, demonstrating a mesial tipping of the maxillary first molar during treatment. It was concluded that the maxillary first molar can be displaced in a variety of directions in achieving successful class II non-extraction treatment. Both the appliance type and the practitioner can have an effect on the displacement of the molar.

ASSOCIATION BETWEEN PREMOLAR EXTRACTIONS AND THE VERTICAL DIMENSION

Shawn Perce[1] and Sean Carlson[2]

Orthodontics Residency[1] and Department of Orthodontics[2], School of Dentistry, University of the Pacific, San Francisco, CA 94115

OBJECTIVE: The present study was undertaken to investigate the influence of premolar extractions on the vertical dimension.

METHODS: In an effort to minimize bias, a "borderline" extraction sample was created via retrospective analysis of the University of the Pacific Orthodontic Clinic Archives. This was done to minimize the inherent differences of two samples with different pretreatment characteristics. The vertical cephalometric changes of 32 patients (22 nonextraction, 10 extraction) were analyzed using a computer aided cephalometric technique to minimize landmark identification error. The following pre and post treatment cephalometric variables were acquired: mandibular plane angle, Y-axis, Overjet, Overbite, upper face height, lower face height, and total face height, maxillary molar position and mandibular molar position. Two tailed student's t-tests were used to examine the differences in pretreatment (T1), and postreatment (T2) lateral cephalometric radiographs. The differences in the change incurred during treatment (T2-T1) between the two groups were also examined via t-tests.

RESULTS: There were no statistically significant differences between the extraction and nonextraction sample with regard to the vertical changes occurring during treatment.

CONCLUSION: These data suggest that premolar extractions do not result in a decrease in a patient's vertical dimension.

CLINICIANS' ESTIMATES OF INCIDENCE OF TOOTH LOSS RELATED TO APICAL ROOT RESORPTION

Vernon W. Pollock[1], Sheldon Baumrind[2], Robert L. Boyd[2], Sean Carlson[2]

[1]Orthodontics Residency Program and [2]Department of Orthodontics, School of Dentistry, University of the Pacific, 2155 Webster Street, San Francisco, CA 94115

Orthodontists are concerned that apical root resorption associated with full-bonded treatment is occasionally of sufficient magnitude to contribute substantially to the loss of one or more permanent teeth. This pilot study is an initial attempt to survey dental clinicians' perception of the incidence of such events in normal clinical practice. In this preliminary survey, a five-item questionnaire was executed by a self-selected sample of 57 practicing dental clinicians attending the 2003 Annual Meeting of the Alumni Association of the UOP School of Dentistry. (Total Years in Practice = 668, Mean = 14.5, Range = 6 to 46.) Embedded among several masking questions was the key inquiry asking how often each practitioner had found it necessary to order the removal of one or more permanent teeth in which the major contributing factor was apical root resorption associated with orthodontic treatment. Masking questions solicited information on the incidence of tooth loss from other causes. Only 15 of the 57 clinicians (~1/4) recalled ever having been required to order permanent tooth removal as a consequence of apical resorption associated with orthodontic treatment. Among the clinicians responding to this query, root resorption associated with orthodontic treatment was only rarely the primary cause of tooth loss in the permanent dentition.

ABSTRACTS

FACULTY

DO REPEATED CLINICAL COMPETENCY RATINGS STEREOTYPE STUDENTS?

David W. Chambers[1]

[1]*Department of Academic Affairs, School of Dentistry, University of the Pacific, 2155 Webster Street, San Francisco, CA 94115*

Stereotypes are judgments that are incomplete summaries, based in part on non-essential characteristics that distort or prevent accurate use of new information. Faculty ratings of student clinical competency over the final two years of the educational program in the University of the Pacific School of Dentistry in areas of clinical judgment, patient management, and technical skill were analyzed. This represented 149 students with eight sets of ratings comprising an average of 16.2 faculty ratings per student. Ratings were classified as P = rating by a faculty member of a student during a particular quarter when the same faculty member also rated the same student the next quarter, N = rating by a faculty member of a student whom that faculty member did not rate the previous quarter, and R = rating by a faculty member of a student in a subsequent quarter after having been rated by the same faculty member the previous quarter. PR correlations across quarters (repeated evaluations by the same instructor) tend to be higher than PN correlations (evaluations of the same student by different faculty members). Using partial correlation (holding new ratings constant) shows that the proportion of experience carried over begins at about 10% at the start of each year and rises to about 20% by the end of first year and to about 50% by the end of second year. However, prediction of graduation quarter competence is significantly better for faculty members who perform repeat evaluations. It is concluded that faculty do carry their knowledge of prior student experience over to subsequent ratings of students, but that this knowledge is more accurate than is the knowledge of first-time faculty raters.

ELECTROKINETICS OF STEALTH® LIPOSOMES

Valentina A. Khorosheva[1] and Joel A. Cohen[1]

Department of Physiology, School of Dentistry, University of the Pacific, 2155 Webster Street, San Francisco, CA 94115

Liposomes are colloidal particles composed of lipids. Liposomes decorated with surface-grafted poly (ethylene glycol) (PEG) polymers, known as sterically-stabilized or Stealth® liposomes, are in current therapeutic use for intravenous delivery of anti-cancer and anti-fungal drugs. Dental applications for oral cancer and antibiotic therapy are being evaluated. The "hairy" polymer coating retards recognition of the liposome by the host immune system, buying valuable time for systemic circulation and therapeutic activity. To elucidate the physical structure of the polymer coats, we measured electrophoretic mobilities of a series of such liposomes, determining their hydrodynamic drag under various conditions. End-grafted PEGs ranged in size from 2 to 113 monomers; nominal PEG grafting densities ranged from one PEG chain per 200 lipids to one PEG chain per 10 lipids; and NaCl ionic strength ranged from 0.5 mM to 100 mM. The liposome surface-charge density was held constant at one negative charge per 10 lipids by appropriate inclusion of charged non-PEGylated lipids. Identically charged liposomes having no grafted PEG served as controls. The observed drag effects are large: they reduce the motilities by up to 90%. The investigated polymer lengths and grafting densities span the mushroom-to-brush transition. The mobilities are analyzed to yield hydrodynamic coat thicknesses, polymer-water frictional coefficients, and polymer segment-density profiles. These studies are of practical significance in the future design of liposomes for drug delivery. (Supported in part by the Pacific Dental Research Foundation)

CHARACTERIZATION OF KANAMYCIN AS A SELECTABLE MARKER FOR MULTICOPY TRANSFORMANTS IN P. PASTORIS

Matthew Hashimoto[1], Christopher Hatae[1], Geoff & Joan Lin-Cereghino[1]

[1]*Department of Biological Sciences, University of the Pacific, 3601 Pacific Avenue, Stockton, CA 95211*

The yeast *Pichia pastoris* is commonly used as a host organism in heterologous protein expression, which is a process that is utilized to produce many pharmaceuticals and other industrial products. One of the main problems of *P. pastoris* is that it does not have a wide range of selectable markers available for use in transformation and expression processes. This being the case, the use of *P. pastoris* can be expensive in that one of the few efficient selectable markers on hand is the zeocin resistance gene. Currently only one company, Invitrogen, distributes a commonly used reporter gene, the zeocin resistance gene. Zeocin is an antibiotic that binds to the DNA of a cell (e.g. *P. pastoris*) and cleaves it causing cell death. In this experiment we will attempt to adapt a kanamycin resistance selectable marker to fulfill the same function of the zeocin resistance selectable marker. By creating a new selectable marker using kanamycin resistance the costs of transformation in lab research will be cut drastically. With this new cassette for a selectable marker we hope to open the market for other researchers enabling them to be economically efficient in the lab.

CELL-SURFACE HEPARAN SULFATE AND HIV-1 INFECTION OF DIFFERENTIATED THP-1 CELLS

K. Konopka[1], A. Lee*[1,2], J. Monzon-Duller[1], and N. Düzgüneş [1]

[1]Department of Microbiology and [2] Doctor of Dental Surgery Program, University of the Pacific, School of Dentistry, 2155 Webster Street, San Francisco, CA 94115

OBJECTIVES: Recently, it has been reported that the cell surface glycosaminoglycan, heparan sulfate (HS), mediates the attachment of HIV to adherent cells expressing low CD4, such as HeLa-CD4 cells or macrophages, prior to virus entry. The established monocytic THP-1 cell line has been used to study HIV-monocyte/macrophage interactions and the relationship between differentiation, virus production and virus latency. Treatment with phorbol myristate acetate (PMA) induces differentiation of THP-1 cells into adherent macrophage-like cells, which are susceptible to M-tropic, CCR5-dependent HIV isolates. Differentiation of THP-1 cells markedly reduces CD4 surface expression *(Konopka & Düzgünes, AIDS Res. Hum. Retroviruses 2002;18:123-131).* Here we examined if HS is involved in HIV binding and infectivity in differentiated THP-1 cells.

METHODS: PMA-treated THP-1 (THP-1/PMA) cells were incubated with either heparinase (heparin-specific) or heparitinase I (HS-specific) for 2 h at 37°C. Expression of HS on the surface of THP-1 cells was examined using a fluorescent Monoclonal Anti-HS (10E4 epitope) antibody. Infection of THP-1 cells with the M-tropic HIV-1_{BaL} isolate and HIV binding were monitored by ELISA determination of p24 antigen in harvested culture supernatants and cell lysates, respectively.

RESULTS: PMA-treatment resulted in an upregulation of HS expression. Over 40% of THP-1/PMA cells tested highly positive for HS expression (Mean Fluorescence Intensity, MFI = 22.5) when compared to 4.0% (MFI=5.4) for THP-1 cells growing in suspension. Treatment with the HS-specific heparitinase I reduced expression of HS by over 60%. Treatment with heparinases, however, did not reduce HIV binding and did not affect HIV infection in THP-1/PMA cells.

CONCLUSIONS: Our results suggest that HIV infection in THP-1/PMA cells may be independent of cell-surface HS.

(This work was supported partially by the Pacific Dental Research Foundation, Grant 521)

AMBISOME INHIBITS THE ADHERENCE OF CANDIDA ALBICANS TO HELA CELLS

K. Konopka[1], B. Dorocka-Bobkowska[2], and N. Düzgüneş [1]

[1]Department of Microbiology, University of the Pacific, School of Dentistry, 2155 Webster Street, San Francisco, CA 94115; [2]University of Medical Sciences, Poznan, Poland

OBJECTIVES: Candidal adherence to epithelial cells is significantly reduced when antifungal polyenes are present during the "adherence phase", but the treatment does not result in detachment of cell-associated yeasts. It has been reported that Candida biofilms with reduced susceptibility to conventional antifungals, are sensitive to lipid formulations of amphotericin B (AMB). Here we examined the effect of AmBisome, the liposomal AMB formulation, and free AMB on the adherence of C. albicans to HeLa cells.

METHODS: The adherence of C. albicans to HeLa cells was determined as described by Samaranayake et al. (1994). Cells were either incubated with Candida in the presence of the drug or pre-incubated with yeasts for 1 hr at 37°C and subsequently exposed to the drug. The cytotoxic effect of the drugs on HeLa cells was determined by an Alamar Blue assay.

RESULTS: AmBisome was not toxic in the range 1 – 256 μg/ml, while AMB was toxic above 4 μg/ml. Following the 1 hr incubation, in the presence of AMB at 1 and 4 μg/ml, the adherence of C. albicans was reduced by 58 and 71%, respectively. Under these conditions, AmBisome at 1, 4, 16, 64 and 256 μg/ml reduced adherence by 54, 63, 70, 76, and 83%, respectively. These values were significantly different from the controls ($P < 0.0005$). The susceptibility of cell-associated Candida to AMB and AmBisome was significantly lower. The reduction in adherence was between 4 and 10%, when compared to the drug-free controls. The values obtained for AmBisome at 16, 64 and 256 μg/ml were significantly different from the controls ($P < 0.05$).

CONCLUSIONS: The liposomal AMB formulation, AmBisome, which is not toxic in a wide range of concentrations, inhibits candidal colonization when present during the "adherence phase", while the cell-associated Candida yeasts are highly resistant to antifungals in terms of adherence.

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CONCLUSIONS: The liposomal AMB formulation, AmBisome, which is not toxic in a wide range of concentrations, inhibits candidal colonization when present during the "adherence phase", while the cell-associated Candida yeasts are highly resistant to antifungals in terms of adherence.

AN EVALUATION OF THE USE OF AN ARTIFICIAL NEURAL NETWORK TO PREDICT THE RISK FOR ADVERSE DRUG REACTIONS FROM LOW-DOSE ASPIRIN CHEMOPROPHYLAXIS

Ninad Patil[1] and Timothy J. Smith[2]

[1]Pharmaceutical and Chemical Sciences Program and [2]Department of Pharmacology and Physiology, T J Long School of Pharmacy, University of the Pacific, Stockton, CA

Artificial Neural Networks (ANNs) are software programs inspired by and designed to simulate the way in which the animal brain is supposed to process information. The "intelligence" of the biological brain arises from its capability for learning, analysis, prediction and recognition. Though the complexity of the biological brain is far greater than the present-day neural network models, ANNs have shown an impressive ability for learning from data sets and detection of non-linear hidden relationships not elicited by conventional statistical methods. Over the last decade, many studies have been published demonstrating the efficacy of ANNs in the biomedical field; especially for the diagnosis and prognosis of diseases, and Pharmacokinetic-Pharmacodynamic modeling.

Our laboratory is evaluating the application of ANNs in the field of adverse drug reactions (ADRs); as to whether the risk of their occurrence in a patient can be predicted from his/her physiological or pathological parameters. Aspirin, at low doses (81-325mg/day), is commonly advised after the age of 50 as a blood-thinning agent for the prophylaxis of coronary heart disease. Gastrointestinal side-effects with its use are of particular concern, especially in the elderly population.

We are currently working on data obtained from a prospective study on patients in a hospitalized geriatric population (>70 yrs), followed over a period of 5 months. The patients were evaluated by two geriatricians and two clinical pharmacists at the start and end points of the study; with respect to drug therapy, medical history, laboratory data and ADRs. A portion of this data was used to train NeuralWorks *Predict*, a commercially available ANN and construct several tentative models for prediction of ADRs to low-dose aspirin. Another subset of this data was kept aside to be used for testing these models.

Preliminary results indicate that a model trained using Blood platelet count (PC), Prothrombin time (PT), Partial Thromboplastin Time (PTT) and Blood glucose levels show some potential for use in predicting the risk of occurrence of ADRs to Aspirin, at the low doses used for chemoprevention. Validation of these ANN models using external data from another hospital is still pending.

FACTORS AT PLAY IN CLEFTING. AN EPIDEMIOLOGICAL STUDY SEARCHING FOR VARIOUS FACTORS CAUSING CLEFT LIP AND PALATE ANOMALIES IN CEBU CITY, PHILIPPINES.

Tripti Pawar[1,2], Miroslav Tolar[2] and Marie Tolarová[1]

[1]Craniofacial Genetics Laboratory, Department of Orthodontics, School of Dentistry, University of Pacific, 2155 Webster Street, San Francisco, CA 94115; [2]Pediatric Clinical Research Center, PCRC Core Laboratory, Children's Medical Center, UCSF, 505 Parnassus Avenue, San Francisco, CA 94143

Cleft lip and palate anomalies, including syndromes and multiple congenital anomalies, affect one out of every 550 newborns in most countries with adequate registries. With the world population on a steady rise, there is an increasing need for research to evaluate the role of both environmental and genetic factors in the etiology of clefts.

Our study population came from the island province of Cebu, Philippines. We looked at 104 individuals affected with orofacial clefts and 76 controls (patients without a congenital anomaly). A spectrum of factors such as sex distribution, age, family history, birth order, age of the mother at the time of birth of the index child, and month of birth were evaluated.

The mean age of patients with an orofacial cleft was 7.9 years, a majority of which (72%) had cleft lip and palate. The mean age for controls was 4.26 years. Among cases, the left side was more often affected (71%) than the right side, a result similar to that reported by others. A family history of clefting was positive in 60% of cases. The mean value for birth order was 3.4. A higher birth order was found in cases affected with bilateral clefts (3.9); however this difference was not statistically significant. The mean maternal age at the time of the child's birth was 27.4 years for cases and 26.1 years for controls. When maternal age was analyzed by two-years age groups, the majority of case mothers (23.91%) fell in the 24-26 years age group, and the majority of control mothers fell in the 18 to 20 years age group.

A three-month weighted average of the month of birth of cases and controls showed two peaks – one for the months of May to July and one for the months of September to November. Projected to the time of conception, these peaks showed that a high number of affected individuals were conceived in the months of September to November (end of the rainy season in the region) and in the period from December to February (the dry season).

This pilot epidemiological study evaluates several variables that are historically considered as risk factors for congenital anomalies. More extensive analysis of environmental, nutritional, and genetic factors is in progress to determine the factors in the etiology of cleft lip and palate in this Southeast Asian region.

UPDATE ON PRIMARY PREVENTION OF CLEFT LIP AND PALATE ANOMALIES

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As many as 700 children are born every day with orofacial cleft in the world. One baby every two minutes. The treatment of cleft anomalies has tremendously improved and many children with a cleft have a normal life. However, with projected population increase, a number of individuals affected with a cleft will increase, if we do not invest in prevention. Our research and studies by others have shown that a significant proportion of nonsyndromic cleft lip and palate (NCLP) can be prevented by periconceptional supplementation with folic acid (FA).

OBJECTIVE: To update a protocol for primary prevention of NCLP.

METHOD: We combined results of our studies and works by others focused on environmental and genetic factors, and mothers' nutrition in relation to NCLP etiology.

RESULTS: Different daily doses of FA administered periconceptionally are needed for prevention of occurrences and recurrences. Our previous studies showed a decrease of recurrences by 65% when multivitamins and 10 mg of FA were daily administered to the mother, and a decrease of occurrences by 27-50% when 400 mcg of FA were daily obtained by the mother from her diet. Mother's diet low in FA and zinc, especially when genetic susceptibility is increased by mutations of candidate genes (C677T mutation of the methylenetetrahydrofolate gene, A80G mutation of the reduced folate carrier 1 gene) increases a risk for having a baby affected with NCLP. A spectrum of environmental and genetic factors was found to differ in each location studied.

CONCLUSION: Detailed data on mother's nutrition, lifestyle, medical and genetic history combined with ascertainment of mutations in candidate genes are suggested for individual prevention protocols, while considering a baseline information from an appropriate population and location.

The studies included in this presentation were supported by Rotaplast International and by The Smile Train/PSEF.

ABSTRACTS

ADA/DENTSPLY STUDENT RESEARCH COMPETITION

IMPACT OF PATERNAL METHYLENETETRAHYDROFOLATE REDUCTASE GENE (MTHFR) 677CT MUTATION ON FACIAL CLEFTS

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Evidence suggests that both genetic and environmental factors play a role in the etiology of cleft lip and palate. It has been long understood that folate metabolism is critical for normal, healthy prenatal development. For some time, the 677CT mutation of the methylenetetrahydrofolate reductase gene (MTHFR) has been considered a genetic risk factor for non-syndromic CL/P. MTHFR plays a crucial role in the folate metabolic pathway and its 677CT variant (leading to an alanine to valine substitution) results in a thermolabile enzyme and decreased production of folate. An individual carrying the mutated allele in homozygotic condition (677TT) has a 50% compromised capacity for processing dietary folate. However, a high-dose of folic acid supplementation may overcome this disadvantage. Therefore, understanding the genetic profile of individuals affected with a cleft - and those unaffected but related to cleft patients - is fundamental for cleft prevention.

In any given individual, the genetic mutation that we are observing can either be inherited from a parent or may occur on its own as a fresh mutation. Historically, research in this area has focused on the mother and affected child.

This pilot study attempts to look at the genotypes of the fathers of affected children and examine the paternal genetic contribution in respect of MRHFR polymorphism.

MATERIALS AND METHODS: The cases for this pilot study were collected during Rotaplast medical missions to Guatemala City, Guatemala in 2001 and 2002. They were fathers of children affected with cleft lip or with cleft lip and palate. We selected 36 families. Blood was drawn, subsequently spotted on filter paper and DNA was isolated from dry blood spots and amplified using Polymerase Chain Reaction (PCR). Polyacrylamide Gel Electrophoresis (PAGE) was used to establish MTHFR 677CT genotypes.

RESULTS: Analysis of paternal genotypes showed that the wild type homozygous CC genotype was present only in 4 (11.1%) of 36 fathers tested, the heterozygous CT genotype in 15 (41.7%) and the homozygous TT genotype in 17 (47.2%). Thus, the proportion of C to T alleles showed that the mutated T allele prevails (68.1%).

CONCLUSION: The results of our pilot study suggest a much stronger paternal genetic contribution of MTHFR 677CT polymorphism to the etiology of non-syndromic clefts in Guatemala than expected. Further study on a larger sample and on a control population is in progress.

SUICIDE GENE THERAPY FOR ORAL CANCER: APOPTOSIS OR NECROSIS?

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Oral squamous cell carcinoma (SCC) is a disfiguring disease. It kills 31,000 people per year in the United States and is still without an efficient, effective cure. Several gene therapy approaches are being explored to treat oral SCC. "Suicide" gene therapy involves the delivery of the Herpes Simplex Virus thymidine kinase (HSV-*tk*) gene to cancer cells followed by the administration of the antiviral drug ganciclovir, which is a nucleoside analog. The viral thymidine kinase monophosphorylates ganciclovir, which is then di- and tri-phosphorylated by cellular kinases. Tri-phosphorylated ganciclovir incorporates into replicating DNA during cell division, and causes chain termination and eventually cell death. While this method has been proven successful in killing cancer cells, the mechanism by which cell death is mediated in oral SCC is not known. The aim of this study was to examine the mode of cytotoxicity, whether it is via programmed cell death (apoptosis) or by necrosis. Human HSC-3 SCC cells were transfected with the plasmid pCMVHSV-*tk*, using the cationic lipid reagent Metafectene, and incubated with or without ganciclovir up to 9 days. Cell viability was ascertained by the spectroscopic Alamar Blue assay on days 3, 6 and 9. The extent of necrosis was demarcated by the entry of the dye, Propidium Iodide, into cells and its ability to stain nuclei red. Apoptosis was ascertained by the binding of fluorescent Annexin V to phosphatidylserine exposed on the outer leaflet of the plasma membrane, resulting in bright green fluorescence. While significant ganciclovir-dependent cytotoxicity was observed, ganciclovir-independent cytotoxicity was also noted. The latter may be attributed to cytotoxicity caused by the transfection procedure. Fluorescence microscopy observations indicated that HSV-*tk* + ganciclovir-mediated cytotoxicity was dominated by necrosis rather than apoptosis.

CHILDREN AFFECTED WITH AN OROFACIAL CLEFT IN JIUJIANG, CHINA AN EPIDEMIOLOGICAL STUDY.

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Cleft lip and palate anomalies that affect approximately one in every 700 newborns worldwide are historically more common in Asian countries. Interestingly, this tendency was not observed when prevalence of orofacial clefts was estimated among Asians who live in California. The purpose of this pilot study was to investigate epidemiological characteristics of children born with a cleft in the city of Jiujiang in the Jiangxi province of China.

The data were collected during personal interviews with mothers of children with a cleft or with adult patients, in October 2002 during a Rotaplast medical mission. Data on 133 patients were analyzed for this study. The patients' mean age was 7.6 years. The oldest patient was 48.3 years old, the youngest was 2 months old. There was a predominance of males in our sample (male to female ratio = 1.82). Patients were classified according to cleft diagnosis into six subgroups: CL (cleft lip), CLP (cleft lip and palate), CP (cleft palate), Sy (syndrome), MCA (multiple congenital anomalies), and unspecified type of orofacial cleft. The vast majority of our patients were affected with CLP (42.85%), followed by CL (23.31%). The mean birth weight of males was 3103.7 g; the mean birth weight of females was 2958.3 g. As we expected, a majority of our patients were first born, because of the one-child policy in China. The mean birth order for male patients was 1.9, and the mean birth order for female patients was 1.6. This difference, which may reflect the preference for boys in Chinese families, was statistically significant ($p= 0.012$). The mothers' mean age at the time of birth of a child with a cleft was 24.7 years. The youngest mother was 9.4 years old, and the oldest mother was 40.3 years old.

Results of this pilot study give us a first, general overview of characteristics of patients affected with orofacial clefts in Jiangxi province. More detailed evaluations of our cleft sample are in progress.

A STUDY OF FAMILIAL AND ENVIRONMENTAL RISK FACTORS FOR CLEFT LIP AND PALATE, IN JIUJIANG, CHINA.

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Studies have shown that congenital anomalies such as cleft lip and palate have multiple and complex risk factors, both genetic and environmental. In this pilot study of 133 families with children with a cleft anomaly, we examined some of the factors affecting the development of cleft lip and palate in the population of Jiujiang, China.

Among the environmental factors studied are: family history of congenital anomalies, birthplace, location of residence, socioeconomic level, occupation of the parents during the index pregnancy, mother's health problems and medication usage during pregnancy, mother's lifestyle, number of children in the family, and availability of prenatal care.

The study confirms our hypothesis that there is an association between cleft lip and palate and a positive family history (36.4%), residence in a rural countryside (62.2%), homebirths (47.4%), and low family income (36%). There is also an association of cleft lip and palate with the mother's health and lifestyle during pregnancy: 52% of mothers indicated they had suffered from a cold sometime during their pregnancy, and 19.8% had taken either cold medicine or antibiotics for that condition. Other specific details are required for further analyses of the type and time of these fetal insults. Also, our results need confirmation in a case-control study.

EPIDEMIOLOGY OF OROFACIAL CLEFTS IN THREE POPULATIONS

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This study is based on 767 individuals from Venezuela (Barquisimeto and Cumana) and Guatemala (Guatemala City). From these three populations the sample is comprised of 515 cases of cleft lip with or without cleft palate (CL±P) and 252 controls (individuals without birth defect).

Each subject was given a thorough physical exam followed by an extensive questionnaire and interview. To evaluate associations of factors contributing to the etiology of CL±P, we analyzed several epidemiological characteristics considered to be involved in causes of congenital anomalies of orofacial region.

From previous studies we have learned that environmental as well as genetic factors are "location specific", i.e. in different locations different factors may play a major role. Analysis of parental age and birth order was undertaken using STATA version 8 software.
This study showed, that birth order of children born with cleft differed significantly between controls and cases in all our three populations ($p=0.006$). Also other characteristics, like parental age, seasonal incidence, place where family lived, and other, showed interesting results.

ULTRASOUNDS: A LITTLE INFORMATION CAN GO A LONG WAY

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Orofacial clefting is a congenital anomaly that occurs as a failure of structural facial processes to fuse during the 5th to 12th week of gestation. Surgical repair is possible, but requires a multidisciplinary approach. Prenatal detection with ultrasounds by well-trained technicians is a beneficial tool that can help prepare parents emotionally and financially for the care of their child. This investigation revealed that the frequency of misdiagnosis of prenatal diagnosis occurs at an alarming rate in the 3 third world countries studied: Guatemala City, Guatemala; Barquisimeto, Venezuela; and Cumana, Venezuela. Mothers of children with cleft lip and/or palate that had an ultrasound during the pregnancy were asked if the congenital anomaly was prenatally diagnosed. Overwhelming, at least 90% of cases studied were diagnosed incorrectly; the highest percentage (98.6%) seen in Guatemala City. The findings warrant further study into the causes of low efficacy of prenatal ultrasound diagnoses third-world countries.

MANDIBULAR FRACTURE SURVEY: A 10-YEAR RETROSPECTIVE AND PROSPECTIVE ANALYSIS

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Retrospective analysis of 10-years accumulation of cases at the Alameda County Medical Center Highland Campus reveals insight into the demographics behind mandibular and oral-maxillofacial trauma. In 52 selected cases, spanning 10 years, a detailed data analysis was gathered assessing various demographic features, such as age, sex, race, disease, substance abuse, mechanism of injury, anatomical location of injury and treatment and follow-up. In a preliminary evaluation on the topics, our study has shown a considerable correlation of trauma with substance abuse, sex and race of the victim. Further investigative evaluations will allow us to better understand the culture behind violence in an underdeveloped and impoverished community.

THE USE OF THE INTRA ORAL CAMERA WITH PEDIATRIC PATIENTS IN A PEDIATRIC DENTAL CLINIC

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The purpose of this study is to examine the effect the addition of the intra oral camera has to the standard protocol on preventive care on the self-care practice of pediatric patients between ages 7 to 14 years of age in a pediatric dental clinic. Two similar patient groups will be studied. Patients from both groups will receive a prophylaxis and topical fluoride from a dental student (supervised by a clinical instructor). The indices to be used are DMFT scores, Modified Gingival Index (Lobene) and OHI-S (Greene and Vermillion), which are currently used for all patients as part of typical treatment protocol in the Pediatric Dental Clinic. At the end of the preventive visit, a questionnaire will be given to the patient and parent to assess the effectiveness of the intraoral camera as an adjunct to preventive care. When the patients return for their 6-month recall visit, the DMFT scores, Gingival Index and Plaque Index will be compared.

DEVELOPING AN ASSAY TO DETECT LIGAND BINDING TO THE COILED COIL DOMAIN OF HIV-1 GP41

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INTRODUCTION: We are developing an assay to test for compounds which bind to the coiled coil domain of the gp41 fusion protein of HIV, and may therefore act as potent antiviral drugs. Currently there is a peptide, enfuvirtide, which has been FDA-approved for treatment, but which suffers from the drawbacks of high cost and requiring parenteral administration. Once developed, our assay will be used to rapidly screen large libraries of non-peptide compounds for binding affinity.

METHOD: There are two components comprising our assay. The first is a peptide similar to enfuvirtide which should bind to the coiled coil and which we label with a fluorophore. The second is a designed gp41 coiled coil, which has attached at its N-terminus a metal coordination center that can absorb light emitted from the fluorophore. The binding event results in quenching of fluorescence, which we monitor using a fluorimeter. Non-peptide compounds, which bind competitively, can then be detected because they will cause the fluorescence to increase.

GOALS: We have carried out experiments to fine-tune the assay to determine (i) an optimal fluorophore for use in the assay and (ii) the specificity of the peptide for the binding site of the coiled coil.

RESULTS: (i) We have determined that the fluorophore Lucifer Yellow has excellent properties in sensitivity and wavelength of emission for our assay. (ii) We have found that peptide binding is not completely specific, but that some non-specific hydrophobic effects play a role in binding.

CONCLUSIONS: Future work will include lengthening the peptide or coiled coil to improve binding specificity or developing constrained peptides that present the correct side-chain conformations into the binding site.

NOVEL TRANSFECTION REAGENTS FOR GENE THERAPY OF ORAL CANCER

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Oral squamous cell carcinoma (SCC) is the most common type of oral cancer: Nine out ten oral cancer malignancies are SCCs. Current therapies of oral SCC are not highly effective, with a 5 year survival rate of about 50% if detected early. Our long-term goal is to develop gene therapy for oral SCC based on the delivery of the Herpes Simplex virus thymidine kinase (HSV/*tk*) gene to SCC cells, followed by ganciclovir treatment to mediate cytotoxicity. As a first step towards testing this strategy in an animal model of oral SCC, the ability of novel transfection reagents to deliver a reporter gene into human and murine SCC cells was investigated. The following transfection reagents were tested: 1) NeoPhectin (cationic cardiolipin-based lipid), 2) JetPEI (linear polyethylenimine), 3) GeneJammer (proprietary polyamine and other components), 4) Trojene (proprietary cationic lipid and a neutral helper lipid), and 5) Metafectene (proprietary cationic lipid). These reagents were complexed with the luciferase-expressing plasmid (pCMVluc) at varying ratios to determine optimal conditions for delivery to human HSC-3 and murine SCC-7 cells. Gene delivery was monitored by the expression of luciferase in cell lysates, expressed in relative light units (RLU)/ml of lysate. For HSC-3 cells, the best reagent was found to be Trojene with an optimal ratio of Trojene:DNA (µl:µg) of 4:1. GeneJammer, NeoPhectin and JetPEI mediated much lower levels of gene expression. For SCC-7 cells only Trojene and Metafectene were examined. Trojene was found to be superior to Metafectene and the optimal reagent:DNA ratio was 2:1 (µl:µg) for both. Future experiments will examine the ability of Trojene to deliver HSV/*tk* to SCC-7 cells and to mediate cytotoxicity in the presence of ganciclovir.

ABSTRACTS

SENIOR RESEARCH COMPETITION

SERUM-RESISTANT HSVTK/GANCICLOVIR GENE THERAPY IN ORAL CANCER CELLS

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OBJECTIVES: Oral Squamous Cell carcinoma (OSCC) is the most prevalent cancer involving the oral cavity and oropharynx. The purpose of this study was to deliver the Herpes Simplex Virus thymidine kinase gene (HSV-*tk*) to HSC-3 human SCC cells, as a "suicide" gene therapy approach. We examined the effect of fetal bovine serum (FBS) on the delivery of a reporter gene and HSV-*tk* by the polyamine reagent, Gene Jammer, and the polycationic liposome, Metafectene.

METHODS: We assessed gene delivery by incubating HSC-3 cells with the plasmid pCMV-luc and measuring luciferase activity in cell lysates. Cells transfected with the HSV-*tk* plasmid were incubated in the absence and the presence of ganciclovir (10 μg/ml) for the indicated periods of time. We used the the Alamar blue assay to determine ganciclovir-specific cytotoxicity to HSC-3 cells mediated by the plasmid pCMV-HSV*tk*. Mock-transfected cells served as controls.

RESULTS: The optimal ratios of the reagents to DNA for delivering the luciferase gene were 4 μl Metafectene:μg DNA and 6 or 12 μl GeneJammer:μg DNA. GeneJammer-mediated luciferase expression was inhibited by about 30% when transfection was performed in the presence of 10% FBS. The delivery of the HSV-*tk* gene by Gene Jammer in the absence and presence of 10% FBS, followed by ganciclovir treatment for 9 days, resulted in 100% and 70% cytotoxicity, respectively. With Metafectene, FBS in the range 10-60% inhibited luciferase gene expression by about 60%. Using HSV*tk*/ganciclovir, 90-100% cytotoxicity was observed in the presence of 0 or 10% FBS. Even in the presence of 60% FBS, Metafectene mediated 50-70% cytotoxicity, compared to controls.

CONCLUSION: Our observations suggest that Metafectene may be useful for the gene therapy of OSCC in animal models. (This work was supported partially by Research Award DRES03-027 from the School of Dentistry).

THE EFFICACY OF ECOTRU AND TRICIDE DISINFECTANTS ON HARD SURFACES OF DENTAL UNITS IN A LARGE DENTAL SCHOOL CLINIC

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Infection control has been one of the most important advances in dentistry in the past thirty years. There are many disinfectants available to dental practitioners to maintain the current standards of infection control. Several studies have examined the efficacy of disinfectants on various hard surfaces. We examined the efficacy of two disinfectants (Ecotru and Tricide) on three hard surfaces of a dental unit in a large dental school clinic. These three surfaces include a bench top adjacent to the patient dental chair, a plastic computer screen used to shield notebook computers from contamination, and the shoulder of the patient dental chair. Bacterial samples were obtained from three surfaces of ten dental units after patients had been dismissed from treatment. These surfaces were then disinfected with either Ecotru or Tricide using the spray-wipe-spray method. Bacterial samples were then taken again. The culture media used were blood agar plates and Hycheck slides containing a tryptic soy agar medium and a DE neutralizing agar medium. These samples were then incubated for 48 hours at 36°C. Bacterial colonies were quantified by placing the colony counts in one of five categories of bacterial density: no colonies, very low, low, medium, and high. Although both disinfectants were effective against bacteria on the dental unit hard surfaces, Ecotru appeared to be a more effective disinfectant than Tricide.

PARASYMPATHETIC VASODILATATION IN RAT SUBMANDIBULAR GLANDS: CONTRIBUTION OF ENDOTHELIUM-DERIVED RELAXING FACTORS

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OBJECTIVES: Endothelial-derived relaxing factors (EDRF's), such as nitric oxide (NO), prostaglandins (PG) and endothelium-derived hyperpolarizing factor (EDHF) play a crucial role in the regulation of blood flow. In the rat submandibular gland (SMG) blood flow is largely under neural regulation, but EDRF 's also participate in the vasodilatation accompanying secretion. The purpose of this study was to determine the relative contributions of NO, PG and EDHF to parasympathetic vasodilatation in the SMG.

METHODS: Male Sprague-Dawley rats (N=19, 350-450 grams) were used. Under chloralose anesthesia, parasympathetic stimulation was delivered via the chorda-lingual nerve at frequencies of 2, 5 and 10 Hz (5-6 V, 2 ms). Laser-Doppler flowmetry was used to measure blood flow (perfusion units) in the presence or absence of inhibitors of NO synthase (L-NAME) and PG synthesis (indomethacin). Blood pressure and salivary flow were also monitored. All data were collected and analyzed using PowerLab software. Differences among treatments were analyzed using a repeated ANOVA followed by post-hoc tests for differences between individual means.

RESULTS: In the absence of inhibitors, SMG blood flow (calculated as an integrated area, minutes x perfusion) was 6,159 ± 4530 at 2 Hz, 15,645 ± 6830 at 5 Hz and 22,418 ± 7660 at 10 Hz ($p<0.01$ 5 Hz vs 2 Hz and 10 Hz vs 5 Hz). L-NAME (300 μg/min, i.v.) partially blocked parasympathetic vasodilatation (45% at 2 Hz, and 30% at both 5 Hz and 10 Hz, $p<0.01$). The addition of indomethacin (1 mg/kg, i.p.) led to a further decrease in vasodilatation ($p<0.05$ L-NAME + indomethacin vs L-NAME) to 65% of controls at 2 Hz, 46% at 5 Hz and 34% at 10 Hz.

CONCLUSIONS: Parasympathetic vasodilatation in the SMG depends, in part, on both NO and PG. EDHF also contributes to the observed vasodilatation, particularly at higher stimulation frequencies.

PROTAMINE ENHANCES TRANSFERRIN-LIPOPLEX-MEDIATED GENE DELIVERY AND HSV-*tk*/GANCICLOVIR-MEDIATED CYTOTOXICITY IN ORAL CANCER CELLS

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Oral squamous cell carcinoma (OSCC) is the sixth most common cancer in the United States. Gene therapy approaches are being used in preclinical studies for the treatment of OSCC. Our laboratory is working on methods to enhance gene delivery to OSCC cells in culture as a first step toward the delivery of the Herpes Simplex Virus thymidine kinase (HSV-*tk*) "suicide gene" in an animal model of the disease. Cationic liposome-DNA complexes (lipoplexes) are a promising non-viral vector for the gene therapy of oral cancer. During gene delivery, it is important to protect DNA from nucleases and to condense it to form a compact complex with cationic liposomes. Protamine is an arginine-rich, natural cationic peptide of MW 4000-4250 that condenses DNA. Transferrin-complexed lipoplexes have been shown to enhance gene delivery to a variety of cells, including HSC-3 human squamous cell carcinoma cells, possibly via binding to transferrin receptors.

We examined whether protamine could enhance gene delivery to HSC-3 cells by Transferrin-lipoplexes. We also investigated whether HSV-*tk*/ganciclovir mediated cytotoxicity in these cells. Lipoplexes were prepared by the complexation of transferrin and protamine with DOTAP/Cholesterol liposomes followed by association of plasmid DNA (pCMVluc) encoding the marker enzyme luciferase. The presence of protamine in the range 0.5-2 microgram per microgram DNA enhanced luciferase expression 3-4 fold, depending on the DOTAP/DNA (+/-) charge ratio. HSV-tk/ganciclovir-mediated cytotoxicity in HSC-3 cells was also enhanced by the association of protamine with transferrin-lipoplexes, increasing the percentage of cells killed from 62% to 100% by the 9th day of the experiment. These results suggest that condensation of DNA by protamine can be useful in both gene delivery and gene therapy applications.